GENETIC VARIATION AND THE ECOLOGICAL PARAMETER "FOOD MEDIUM" IN CAGE POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Impressive effects of the ecological factor "food medium" upon the frequencies of the lethal-bearing second chromosomes in *Drosophila melanogaster* were found in cage populations originated from a common gene pool. Allozyme frequencies for two second enzyme loci were also determined. The lethal frequencies found were higher when the environment was restrictive than when it was favorable. Moreover, the lethal frequencies were higher in populations of smaller size than in those of greater. The role of the lethal genetic variants in the structure of populations is discussed.

Des effets marqués du facteur écologique "milieu alimentaire" sur la fréquence des chromosomes No. 2 porteurs de gènes létaux furent observés chez des populations encagées de *Drosophila melanogaster*, originant d'un fond génétique commun. Les fréquences des allozymes pour deux loci situés sur le deuxième chromosome furent également déterminées. Les fréquences des gènes létaux étaient supérieures en milieu restrictif et chez les populations de faibles dimensions. Le rôle des variants génétiques létaux dans la structure des populations est discuté.

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Introduction

The frequencies of lethal and semilethal genetic variants in natural populations of *Drosophila melanogaster* have been correlated with such ecological factors as temperature and rainfall (Band and Ives, 1961, 1968). The fluctuations of the frequencies of these genetic variants in natural populations in relation to the immediate environment indicate that they play a more positive adaptive role in population structure than the frequency of any one individual genetic variant (Band, 1971a,b). However, such associations involve subjectivity, and experimentation of this sort may be more appropriate in laboratory cage populations since the parameter under study can be controlled. Therefore, the selectional role of the ecological parameter "food medium" upon the frequency differentiation of the lethal-bearing second chromosomes was studied in cage populations of *Drosophila melanogaster*.

The maintenance of genetic variants in populations has been related to their heterotic effect (Mukai and Burdick, 1956) and to linkage with heterotic inversions or epistatic gene complexes (Oshima, 1965, 1967, 1968; Watanabe and Oshima, 1966). Thus, we examined isolated second chromosomes (lethals and nonlethals) with respect to their allozyme alleles at the α-Glycerophosphate dehydrogenase (*α-Gpdh*) and Alcolol dehydrogenase (*Adh*) loci. These two enzyme loci were found to associate nonrandomly with the polymorphic inversions of the second chromosome in the cage populations which were examined. Hence, they can be considered as markers of large blocks of genes.

Materials and Methods

The strains of *Drosophila melanogaster* used in this study were derived from adults collected in the Greek island of Cephalonia during the summer 1973. Six (3 females, which were collected and maintained as virgin, and 3 males) 4-6 days old individuals from each of 100 isofemale lines (300 virgin females and 300 males) were allowed to mate randomly in a cage

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(1B). After ten generations, four cage populations ($1B_1$, $1B_2$, $1B_3$, $1B_4$) were derived, by replication, from the original population (1B). Consequently, we may regard the four derived populations as possessing practically the same gene pool at their origin. All these populations were maintained simultaneously at 25± 0.5°C and a relative mean humidity 43±4%. The populations were maintained in a dark/light cycle of 12 h. and were kept in plastic cages the dimensions of which were 41 × 41 × 16 cm and contained 14 vials each (the dimension of vials were 10 × 12.5 cm). Populations $1B_1$, $1B_2$ were kept in the dead-yeast-sugar-agar (10g dead-yeast, 10g sugar, 3g agar per 100 ml H_2O) food medium to which propionic acid was added; while populations $1B_3$, $1B_4$ in the cornmeal sugar-agar (12.5 g cornmeal, 1.8 g sugar, 1 g agar per 100 ml H_2O, as well as 1 ml of diluted living yeast suspension per food vial) where again propionic acid was added (in order to prevent possible growth of yeast). Care was taken to have more or less the same amount of medium in all vials ($^1/_3$ of each vial) used for the experiments. The isolation of second chromosomes was based on the *Cy-pm* inversion method (Auerbach, 1962). Thirty five generations after the origin of the cage populations adult males were extracted from each population and the frequencies of nonlethal and lethal-bearing second chromosomes and allozyme alleles of the *α-Gpdh* and *Adh* loci were determined. The electrophoresis techniques are discussed in detail elsewhere (Alahiotis, 1975b).

Results and Discussion

It is well known that yeast is the primary food source of *Drosophila* (Sang, 1949); moreover, yeast-culture media are very suitable for *Drosophila* culture (Demerec, 1965). Therefore, the cornmeal-sugar-agar food medium can be considered as a poor medium while the dead-yeast-sugar-agar as a rich one. The sizes of the populations ($1B_1$, $1B_2$, $1B_3$, $1B_4$) were at the beginning of their origin the same, that is they had approximately the same number of flies (about 2,800) with the 1B population. It seems that the usable amount of food (mainly in yeast and sugar) per individual fly was very little in the $1B_3$, $1B_4$, populations, while it was enough in the $1B_1$, $1B_2$ ones. Thus, we could assume that the environment of the $1B_3$, $1B_4$ was restrictive; the populations were maintained under crowded conditions and their density was therefore high. In contrast, it may be considered that the $1B_1$, $1B_2$ populations were maintained in a favorable

TABLE I

Frequencies of lethal-bearing chromosomes in the cage populations $1B_1$, $1B_2$, $1B_3$ and $1B_4$

Populations	Frequency	Chromosomes examined	χ^2_1	P
$1B_1$	0.2643	140	$1B_1/1B_4$ 4.256	<.05
$1B_2$	0.2546	110	$1B_1/1B_3$ 6.160	<.05
$1B_3$	0.4041	146	$1B_2/1B_4$ 6.038	<.05
$1B_4$	0.3900	100	$1B_2/1B_3$ 4.401	<.05

TABLE II

Frequencies of the *α-Gpdh*^{F} and *Adh*^{F} allozymes in the $1B_1$, $1B_2$, $1B_3$ and $1B_4$ cage populations

Populations	Frequencies	
	α-Gpdh^F	*Adh*^{F}
$1B_1$	0.6818	0.9379
$1B_2$	0.7058	0.9900
$1B_3$	0.9621	0.6956
$1B_4$	0.9594	0.6333

environment at intermediate (optimal) density. As the main factors limiting the population size are their limiting space and food (Ayala, 1967; Parsons, 1973) the final size of the poor food medium treatment populations ($1B_3$, $1B_4$) was smaller (about 1,100) than that of the rich ones ($1B_1$,$1B_2$) which remained the same as at the beginning. One could say that the progressive decrease of population sizes ($1B_3$,$1B_4$) results in the reduction of their density. In fact, the population density must not be so high as at the beginning, but we think that these populations are maintained under crowded conditions for the following reasons: a) The usable amount of food per individual fly in the final population size is still relatively small, while, in the rich food treatments, a sufficient amount of food has not been consumed after the removal of the food vials from the cage, b) The continuous oviposition increases the competition in the poor food medium treatments, while the rich diet decreases considerably the restrictiveness, and c) It seems to us that the dramatic changes observed in the allozyme frequencies of the *α-Gpdh* and *Adh* loci during 30 generations in the $1B_3$,$1B_4$ populations may better be attributed to the contribution of the factor of competition. In favor of the previously mentioned rationale is the fact that no changes, from the initial (equilibrium) frequencies were observed in the $1B_1$,$1B_2$ populations (Alahiotis and Pelecanos, 1976).

Our results are collected in three tables. Tables I and II show the frequency of lethal chromosomes, as well as that of the *α-Gpdh*^{F} and *Adh*^{F} allozymes. Impressive differences between the two series ($1B_1$,$1B_2$ *versus* $1B_3$, B_4) of cage populations were found. The observed frequencies of lethal bearing chromosomes are higher in the populations $1B_3$ and $1B_4$ than in $1B_1$ and $1B_2$ (Table I). In order to determine whether the differences found are significant a contingency chi-square analysis was performed according to Workman and Niswander (1970; Table I).

An attempt was also made to detect any correlation in the frequencies of the allozymes of *α-Gpdh*, *Adh* loci and the nonlethal and lethal chromosomes. Table III shows that among eight such comparisons there are no associations.

Our findings, we thinks, provide enough evidence to allow the following assumptions:

1. The observed changes in frequencies of lethal-bearing chromosomes and of the allozymes in populations $1B_3$ and $1B_4$ with respect to populations $1B_1$ and $1B_2$ can not be attributed to the random genetic drift (for more details about allozyme frequencies differentiation see Alahiotis, 1975a).

TABLE III

Associations between the allozymes of *α-Gpdh* and *Adh* loci and lethal and nonlethal-bearing chromosomes

Population	locus	Gametic array* IF	IS	NF	NS	Chromosomes examined	χ^2
	α-Gpdh	24	10	66	32	132	0.122
$1B_1$							
	Adh	33	0	88	8	129	2.932
	α-Gpdh	15	9	57	21	102	0.989
$1B_2$							
	Adh	23	0	77	1	101	0.298
	α-Gpdh	53	0	74	5	132	3.486
$1B_3$							
	Adh	35	12	45	23	115	0.902
	α-Gpdh	29	1	42	2	74	0.067
$1B_4$							
	Adh	18	5	20	17	60	3.579

*1 and N refer to lethal and nonlethal-bearing chromosomes and F,S to the enzyme alleles.

2. In these populations a process through which selection acts upon coadapted blocks of genes have been detected (Alahiotis, 1975a; Alahiotis *et al.*, 1975). Allozyme and gene arrangement frequencies were used as markers of this interaction of the gene pool. In addition, linkage disequilibrium was detected between allozymes of the *α-Gpdh, Adh* loci and the polymorphic inversions *In (2L)22D-34A, In (2R)52A-56F*. These changes were dramatic in populations $1B_3$ and $1B_4$ under crowded conditions (poor food medium), while no changes were observed from the initial (equilibrium) frequency in optimum conditions ($1B_1$,$1B_2$; rich food medium). Moreover, although we found no association of lethal frequencies with allozymes (or gene arrangement), it is possible that the observed differences in lethal frequencies reflect the same process of coadaptation of the gene pool. Hence, from this point of view the contribution of the lethal genetic variants in the populations structure is quite possible.

3. Lethal frequencies are frequently used as a relative measure of population size, in such a manner that frequencies are higher in large populations than in small ones (Wright *et al.*, 1942; Ives, 1945; Dubinin, 1946). Thus, Dubinin (1946) has correlated the lethal frequencies with the size of natural populations in *Drosophila pseudoobscura*. In this study the $1B_1$ and $1B_2$ populations are larger than those of $1B_3$,$1B_4$ (Alahiotis, 1975a). However, the lethal frequencies are higher in the $1B_3$ and $1B_4$ populations. Our data favor the view that observed lethal frequencies are largely dependent on nutritional conditions of the populations. Furthermore, if seems that the genetic load is higher in adverse environments than in favorable ones (Tobari, 1966).

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